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## **Title: Tricaine Methanesulfonate (MS-222) Preparation and Use**

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### I. Purpose:

Tricaine Methanesulfonate (MS-222) is a popular anesthetic agent used in aquatic species, and is intended for the temporary immobilization of fish, amphibians, and other aquatic, cold-blooded animals. It has long been recognized as a valuable tool for the proper handling of these animals during manual spawning (fish stripping), weighing, measuring, marking, surgical operations, transport, photography, and research. This document provides guidance on the accepted method of preparation and use of MS222.

### II. Policy:

**Uses:** MS-222 is appropriate for the temporary immobilization of fish, amphibians, and other aquatic, cold-blooded animals.

Pharmaceutical grade MS-222 working solutions must be made up fresh for each use and must be **buffered** for all intended uses on live aquatic species.

### III. Procedure:

The 2020 American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals *recommends to prepare a 10 g/L buffered stock solution with sodium bicarbonate with a pH of 7.0 to 7.5.*

A publication from "The Progressive Fish-Culturist Journal" testing the effects of MS-222 on pH and the buffering capacity of sodium bicarbonate in waters of various hardness show that a ratio of one part MS-222 to two parts sodium bicarbonate buffers the solution to between pH 7.0 - 7.5.

#### MS-222 PREPARATION:

If possible, work inside a fume hood while wearing gloves, goggles, and a lab coat to prepare a concentrated stock solution by mixing one part MS-222 with two parts sodium bicarbonate in proper volume of water and stir to dissolve (e.g. 10 g/L MS-222 and 20 g/L sodium bicarbonate= 1% solution). If fume hood or Engineering Controls are unavailable, use appropriate Personal Protective Equipment (PPE) to work with the powder safely. Subsequently and just before use, dilute the stock solution further as required. Contact Occupational Health and EH&S for guidance on exposures and disposal.

*The stock solution should be stored in a dark brown bottle or covered to make the container opaque as it is light sensitive, and refrigerated or frozen if possible. The stock solution has been shown to be stable at 4°C or -20°C for up to 6 months. The solution should be replaced when it expires at 6 months after reconstitution and any time a brown color is observed.*

Ref: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7338872/>.

#### SOURCE OF PHARMACEUTICAL GRADE MS-222

Syndel's (formerly Western Chemical, Inc.) TRICAINE-S (MS-222, TMS, tricaine methanesulfonate) is an FDA approved fish anesthetic (FDA ANADA 200-226). Company's product Weblink: <https://www.syndel.com/product/tricaine-s-ms-222/>

#### DIRECTIONS FOR USE:

MS-222 is effective and safe for the anesthesia of fish when used as directed. The action of the anesthetic is slowed at cooler temperatures, in extremely soft water (approximately 10 mg/liter of CaCO<sub>3</sub> or less), and in larger fish. Additionally, efficacy may vary with size, species, and concentration of fish. Thus, it is imperative that preliminary tests of anesthetic solution be made against small numbers of fish to determine the desired rates of anesthesia and exposure times for the specific lots of fish under prevailing conditions. MS-222 is also an acceptable method of euthanasia for finfish and for some amphibians and reptiles. When used for large finfish and some amphibians (e.g., *Xenopus* spp.), a secondary method should be used to ensure death (e.g. pithing with or without decapitation).

#### METHODS OF APPLICATION:

1. **General anesthesia.** For most situations where rapid or moderately rapid anesthesia is required, MS-222 may be applied in a bath, e.g., the fish are immersed in the anesthetic solution. Containers may be of glass, plastic, steel, aluminum, or other suitable material. However, do not use galvanized or brass containers unless treated or sealed to prevent dissolution of zinc. The size of container should be determined by individual needs, but the fish should not be overcrowded. To preserve and maintain anesthetic properties, MS-222 working solutions must be discarded after each use event. MS-222 must also be discarded in the following situations:
  - a. Moving animals from one body of water to another
  - b. A sick or abnormally behaving animal has been anesthetized in the solution
  - c. The solution becomes fouled
  - d. The solution becomes cloudy or discolored

**2. Surgery and certain physiologic studies.** The fish may be anesthetized to loss of reflex, removed from the anesthetic solution and then positioned so that the gills are bathed in a sedating concentration of MS-222. Some investigations have developed flowing, recirculating systems for bathing the gills with anesthetic during surgery. Large fishes such as sharks and rays are anesthetized within minutes by spraying the gills with a 1 g/liter solution of MS-222. The application is made by means of a water pistol, bulb syringe, hand pump, etc.

**3. Animal transport.** MS-222 has been used to sedate fish during transport. It is more successful in cold water, and it is instrumental in reducing injuries because of hyperactivity. Fish are usually transported by means of distribution units (tank trucks), or by air in plastic bags. In either case, the fish should be fasted beforehand to reduce metabolic wastes. Additionally, some workers suggest pre-transport sedation for several hours to lower metabolism. With distribution units, the fish may be fasted and sedated prior to loading. The anesthetic solution is prepared in the distribution unit and oxygenated. Then, the fish are added and temperature acclimated.

**4. Dosage use.** Syndel has incorporated a working spreadsheet on their website to help with determining dosages. Weblink:

[https://syndel.com/wp-content/uploads/2019/01/new-tricaine\\_s\\_dosage\\_chart..pdf](https://syndel.com/wp-content/uploads/2019/01/new-tricaine_s_dosage_chart..pdf)

The American Fisheries Society - Fish Culture Section's Working Group on Aquaculture Drugs, Chemicals, and Biologics (WGADCB) has published a Guide to Using Drugs, Biologics and Other Chemicals in Aquaculture. WGADCB have also developed a Treatment Calculator to assist in the dosage calculations of certain products. Weblink: <https://fishculture.fisheries.org/working-group-on-aquaculture-drugs-chemicals-biologics/wgadcb-resources-tools/guide-to-using-drugs-biologics-and-other-chemicals-in-aquaculture/>

The Guide is provided as an Adobe PDF file and the Treatment Calculator is in both [Excel 97-2003](#) (.xls) and [Excel 2010](#) (.xlsx) formats.. Your computer must have installed either Microsoft Excel or any other program that will read and execute calculations of an Excel file. The Guide to using drugs, biologics and other chemicals in aquaculture and Treatment Calculator can be found by clicking the Weblink below:

<https://drive.google.com/file/d/0B43dblZIJqD3Q2NqQkhfeV84emc/view?usp=sharing>

**5. MS-222 euthanasia and proper secondary form of euthanasia of amphibians, reptiles and fish.**

Please refer to the IACUC policy "Guidelines for Euthanasia of Research and Teaching Animals" at: <https://research.ucdavis.edu/policiescompliance/animal-care-use/iacuc/guidelines-for-euthanasia-of-research-and-teaching-animals/>

**6. Precautions during animal use:**

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- Always conduct preliminary tests with MS-222 to determine desired rates of anesthesia and optimal length of exposure.
- Do not contaminate eggs or sperm with MS-222 when stripping fish.
- Do not use water containing chlorine, or other toxic agents to prepare MS-222.
- Treated fish destined for human consumption, must be held in fresh water above 10°C. (50°F.) for 21 days before use.
- MS-222 must be buffered as it drastically decreases the pH of water, which may be toxic and distressful to fish. It has been demonstrated that unbuffered solutions significantly induce skin and corneal ulceration, impact histologic changes, and disrupts blood acid-based balance and hematologic values.

## 7. Storage and Expiration dates:

MS-222 powder = See expiration date on bottle

Stock solution = 4 weeks from the date of preparation when stored at 4°C or when degradation of solution has been seen. (i.e. brown color occurs).

Concentrated stock Solutions = should be stored at -20°C, and should list the expiration date of the powder from the original bottle. A 10 % solution stored at room temperature shows no significant loss of potency after three days, but after 10 days, a brownish color and about 5% decrease in activity is observed.

## 8. MS-222 Disposal

MS-222 solution waste must be disposed of through EH&S hazardous disposal services or another acceptable method. UC Davis Wastewater Treatment Plant does not want this product in the water system. For questions, additional information or waste pick up please contact: Hazardous Waste Management at 530-754-5058; E-mail at [hazwaste@ucdavis.edu](mailto:hazwaste@ucdavis.edu). WebLink: <https://safetyservices.ucdavis.edu/article/waste>

## 9. Safety:

Some good examples of safe practices when working with MS-222 :

- Wear protective clothing, gloves and goggles when handling the MS-222 powder.
- Work inside a fume hood to prepare the solution and stir until all powder is dissolved.
- Collect Tricaine in a bottle or request a hazardous waste container from EH&S. Use [WASTE](#) to create and place hazard and chemical labels with the start of accumulation date, chemical name, and concentration and put labelled container in a double container to avoid spills Use [WASTE](#) to request pick up or an automatic pick-up will be generated after 9 months.
- Wear gloves to handle animals exposed to MS-222.

- Do not discard MS-222 directly into sink, surface water, storm water conveyances, or catch basins.

## 10. References:

- a. American Veterinary Medical Association Guidelines for the Euthanasia of Animals: 2020 Edition: <https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf>
- b. Allen JL, Harmon PD. Control of pH in MS-222 anesthetic solutions. *Progr Fish culturist* 1970; 32:100. Weblink: <http://www.tandfonline.com/doi/pdf/10.1577/1548-8640%281970%2932%5B100%3ACOPIMA%5D2.0.CO%3B2?needAccess=true>
- c. Recommendations for euthanasia of experimental animals: Part 1. DGXI of the European Commission. *Lab Anim.* 1996 Oct;30 (4):293-316. Weblink: [http://journals.sagepub.com/doi/abs/10.1258/002367796780739871?url\\_ver=Z39.88-2003&rfr\\_id=ori:rid:crossref.org&rfr\\_dat=cr\\_pub%3dpubmed](http://journals.sagepub.com/doi/abs/10.1258/002367796780739871?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed)
- d. Recommendations for euthanasia of experimental animals: Part 2. DGXT of the European Commission. *Lab Anim.* 1996 Oct;30 (4):293-316. Weblink: <http://journals.sagepub.com/doi/pdf/10.1258/002367797780600297>
- e. Evaluation and Refinement of Euthanasia Methods for *Xenopus laevis*. *JAVMA*. Vol. 48, No. 5, September 2009. Pages 512-516. Weblink: <https://www.ncbi.nlm.nih.gov/pubmed/?term=Evaluation+and+Refinement+of+Euthanasia+Methods+for+Xenopus+laevis>
- f. Syndel (former Western Chemical Inc.): <https://syndel.com/>
  - 1) Tricaine Safety Data Sheet (SDS): [https://syndel.com/wp-content/uploads/2019/01/tricaine\\_s\\_sds.pdf](https://syndel.com/wp-content/uploads/2019/01/tricaine_s_sds.pdf)
  - 2) Vet Label Product Information: <https://vetlabel.com/lib/vet/meds/tricaine-s/>
  - 3) Tricaine directions for use: <https://syndel.com/wp-content/uploads/2019/01/Tric-S-Insert-101118-FINAL-updated-per-email.pdf>
  - 4) Tricaine dosage chart: [https://syndel.com/wp-content/uploads/2019/01/new-tricaine\\_s\\_dosage\\_chart..pdf](https://syndel.com/wp-content/uploads/2019/01/new-tricaine_s_dosage_chart..pdf)